Instructions for Use

DiaSource®

# Acetylcholine Receptor Autoantibodies (ARAb) RRA

**Radio receptor assay for the semi-quantitative determination of autoantibodies against the acetylcholine receptor in human serum and plasma.**

REF **KIPIB21021**

Σ **100**

2°C 8°C

EU: IVD CE 2797


**IBL International GmbH**
Flughafenstrasse 52a
22335 Hamburg, Germany

Tel. +49 (0) 40 53 28 91-0
Fax +49 (0) 40 53 28 91-11
IBL@tecan.com
www.tecan.com/ibl

**DIAsource ImmunoAssays S.A.**
Rue du Bosquet, 2
1348 Louvain-la-Neuve, Belgium

Tel: +32 (0) 10 84 99-11
Fax: +32 (0) 10 84 99-91
customer.service@diasource.be
www.diasource-diagnostics.com

## REVISION HISTORY OF INSTRUCTIONS FOR USE

| Changes from the previous version 2020-02 to actual version 2023-08 | |
|---|---|
| Cover page | Layout change |
| Chapter 2 | Additional chapter |
| Chapter 3 | Update to scientific validity |
| Chapter 5 | Additional information |
| Chapter 6 | Additional information |
| Chapter 7 | Layout change |
| Chapter 8 | Update / Additional information |
| Chapter 9 | Additional information |
| Chapter 14 | Additional information |
| Chapter 17 | Update |
| Chapter 18 | Update and additional data |
| Chapter 19 | Update |
| Symbol page | Update |

## 1. INTENDED USE

Radio receptor assay for the semi-quantitative determination of autoantibodies against the acetylcholine receptor in human serum and plasma. The ARAb Assay kit is useful as an aid in the differential diagnosis of Myasthenia gravis (MG).

## 2. INTENDED PURPOSE

The Acetylcholine autoantibody radio receptor assay is intended for the semi-quantitative determination of autoantibodies against the acetylcholine receptor in adult human serum and EDTA plasma.
The measurement of highly specific autoantibodies against the acetylcholine receptor (AChr) is appropriate as an aid for diagnosis of myasthenia gravis (MG), a rare but long-term muscle disease leading to muscle weakness of varying severity. In diagnostics, the determination of autoantibodies against the acetylcholine receptor is used as the first screening tool in patients with suspected Myasthenia Gravis disease, as in around 80 % of MG patients autoantibodies against AChR are detectable (seropositive MG). Furthermore determination of autoantibodies provides information concerning (patho-) physiological state of the patient. For definition of myasthenic subgroup, such as early-onset MG, late-onset MG, thymoma-associated or MuSK-associated MG, further diagnostic investigation is necessary. Subgroups will not be defined using this assay. Acetylcholine receptor from human muscle is used as antigen in this radio receptor assay. The receptors are labelled with $^{125}$I-alpha-bungarotoxin, which binds the receptors most specifically and almost irreversible. Autoantibodies present in the patient´s serum or plasma attach to the labelled receptors. The resulting immune complexes are precipitated with anti-human IgG. The amount of radioactivity of the sediment is directly proportional to the concentration of acetylcholine receptor autoantibodies of the sample.
The assay requires general purpose laboratory instruments and consumables such as gamma counter, vortexer and pipettes to execute the test. The manual kit assay test instructions must be strictly adhered to and verified by the laboratory. Test results are calculated from a standard curve and compared to defined cut-off.
The test kit is intended for professional laboratory use by trained personnel.
The test kit is not for home or layperson use.

## 3. SUMMARY AND EXPLANATION

The measurement of highly specific autoantibodies against the acetylcholine receptor (AChr) supports the diagnosis of *myasthenia gravis* (MG), a rare but long-term muscle disease leading to muscle weakness of varying severity. The muscles of the eyes, face and for swallowing are most commonly affected. In diagnostics, the determination of autoantibodies against the acetylcholine receptor is used as the first serological tool in patients with suspected *myasthenia gravis* disease.[1-3] AChR antibodies are directly pathogenic through crosslinking of AChRs following accelerated receptor degradation. Furthermore pathogenic function by inducing AChR conformational changes or blocking acetylcholine binding can be observed.[1]

A distinction is made between several forms of *myasthenia gravis*, for example generalized *myasthenia gravis* or ocular *myasthenia gravis*. Autoantibodies against AChR are detectable in serum of at least 80 % of patients with generalized *myasthenia gravis*[2,4] and in 50 - 60 % of patients with ocular myasthenia.[5] D-penicillamine can cause anti-AChR and anti-MuSK antibody-positive MG, a rare phenomenon, which is reversed after discontinuation of D-penicillamine treatment.[6,7] The examination of patients with suspected MG disease is usually performed with multiple antibody determinations. In the case of AChR seronegativity, the presence of other antibodies such as those against MuSK or Lrp4 can support diagnosis of an existing MG disease in AChR-seronegative patients.[8]

The most sensitive and specific AChR antibody test is a radioimmunoassay (binding antibody assay) using human AChR. It has been the gold standard for MG diagnosis for many years due to its high specificity and sensitivity.[9,10]

## 4. TEST PRINCIPLE

Acetylcholine receptor from human muscle is used as antigen in this radio receptor assay. The receptors are labelled with $^{125}$I-alpha-bungarotoxin. This snake venom binds the receptors most specifically and almost irreversible. Autoantibodies present in the patient´s serum attach to the labelled receptors. The resulting immune complexes are precipitated with anti-human IgG. The amount of radioactivity of the sediment is directly proportional to the concentration of acetylcholine receptor autoantibodies of the sample.

## 5. WARNINGS AND PRECAUTIONS

1. For *in-vitro diagnostic* use only. For professional use only.
2. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
3. In case of severe damage of the kit package please contact DiaAsource or your supplier in written form, latest one week after receiving the kit. Do not use damaged components in test runs, but keep safe for complaint related issues.
4. Broken glass may cause injury. Handle glass vessels with caution.
5. Obey lot number and expiry date. Do not mix reagents of different lots. Do not use expired reagents.
6. Follow good laboratory practice and safety guidelines. Wear lab coats, disposable latex gloves and protective glasses where necessary.
7. Reagents of this kit containing hazardous material may cause eye and skin irritations. See MATERIALS SUPPLIED and labels for details. Safety Data Sheets for this product are available on the DiaAsource-Homepage or upon request.
8. Chemicals and prepared, used, unused or expired reagents have to be treated as hazardous waste according to national biohazard and safety guidelines or regulations.
9. The cleaning staff should be guided by the professionals regarding potential hazards and handling.
10. All serious incidents that have occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.
11. This kit contains radioactive material, to be received, acquired, possessed and used by physicians, laboratories or hospitals only according to regulations and a specific license issued by the Nuclear Regulatory Commission or issued by a state with which the Nuclear Regulatory Commission has entered into an agreement for the exercise of regulatory authority.
12. Radioactive materials should be confined to specifically designated, regularly monitored areas in the laboratory, restricted to authorised personnel. Use disposable labware and disposable absorbent bench covers. Always wear film budges, lab coats and disposable gloves. Wipe up all spills immediately, cleaning the contaminated area with a decontaminant and dispose the contaminated materials as radioactive waste.
13. This kit contains $^{125}I$ (half-life: 60 days), emitting ionizing X (28 keV) and γ (35.5 keV) radiations.
14. The device contains material of animal origin, which is certified apparently free of infectious or contagious diseases and injurious parasites. Still the material should be handled with extreme caution.
15. All reagents of this kit containing human serum or plasma have been tested and were found negative for anti-HIV I/II, HBsAg and anti-HCV. However, a presence of these or other infectious agents cannot be excluded absolutely. For this reason reagents should be treated as potential biohazards in use and for disposal.
16. Some reagents contain sodium azide ($NaN_3$) as preservatives. In case of contact with eyes or skin, flush immediately with water. $NaN_3$ may react with lead and copper plumbing to form explosive metal azides. When disposing reagents, flush with a large volume of water to avoid azide build-up.

## 6. STORAGE AND STABILITY

The kit is shipped at ambient temperature and should be stored at 2 - 8°C. Keep away from heat or direct sunlight. The storage and stability of specimens and prepared reagents is stated in the corresponding chapters.

All kit components are stable up to the indicated expiry date after the kit is opened. Make sure that the bottles are closed with their screw caps and the kit is stored at 2 - 8°C.

## 7. SPECIMEN COLLECTION AND STORAGE

**Specimen**
Serum and plasma (EDTA)

**Specimen collection**
The usual precautions for venipuncture should be observed. It is important to preserve the chemical integrity of a blood specimen from the moment it is collected until it is assayed. Do not use grossly hemolytic, icteric or lipemic specimens. Samples appearing turbid should be centrifuged before testing to remove any particulate material.

**Specimen storage**
Samples can be stored at 2 - 8°C for 7 days
It is recommended to freeze samples and store at -20°C for long time storage (< 6 months).
Avoid repeated freeze-thaw cycles. Keep away from heat or direct sunlight.

## 8. MATERIALS SUPPLIED

| KIPIB21021 | Symbol | Origin | Component |
|---|---|---|---|
| 3 x 3.6 mL | TRACER LYO | | **ARAb$^{125}$I-Tracer** lyophilized<br>Activity: < 100 kBq (2.7 µCi)<br>Contains fetal calf serum, $^{125}$I alpha-bungarotoxin labelled human Acetylcholine Receptor and ≤ 0.1 % sodium azide (w/w). |
| 1 x 22 mL | ANTISERUM | | **IgG Antiserum** Ready to use.<br>Contains goat anti-human IgG and ≤ 0.1 % sodium azide (w/w). |
| 1 x 1.5 mL | CAL A | | **Standard A** = Sample Diluent Ready to use.<br>0 nmol/L<br>Contains: Human serum and ≤ 0.1 % sodium azide (w/w). |
| 1 x 5 x 0.2 mL | CAL B-F | | **Standard B-F** Ready to use.<br>0.2; 0.5; 1.2; 3.0; 8.0 nmol/L<br>Contains: Human serum, antibodies to acetylcholine receptors and ≤ 0.1 % sodium azide (w/w). |
| 1 x 0.2 mL | CONTROL CO | | **Cut-Off Control** Ready to use.<br>Contains: Human serum, antibodies to acetylcholine receptors and ≤ 0.1 % sodium azide (w/w).<br>Concentrations / acceptable ranges see QC certificate. |
| 1 x 0.2 mL | CONTROL + | | **Positive Control** Ready to use.<br>Contains: Human serum, antibodies to acetylcholine receptors and ≤ 0.1 % sodium azide (w/w).<br>Concentrations / acceptable ranges see QC certificate. |
| 2 x 110 mL | WASHBUF | | **Wash Buffer** Ready to use.<br>Phosphate buffer containing ≤ 0.01 % Triton X-100 and ≤ 0.1 % sodium azide (w/w). |
| 1 x 12 mL | BUF | | **Buffer** (For reconstitution of the Tracer.) Ready to use.<br>Phosphate buffer containing ≤ 0.5 % Triton X-100 and ≤ 0.005 % sodium azide (w/w). |

## 9. MATERIALS REQUIRED BUT NOT SUPPLIED

1. Micropipettes (Multipette Eppendorf or similar devices, < 3 % CV). Volume: 20, 100, 200, 1000 µL
2. Round-bottom plastic test tubes (12 x 75 mm)
3. Rack for test tubes
4. Decanting rack or aspiration system
5. Vortex mixer
6. Centrifuge (preferably refrigerated); ≥ 2000 x g
7. Gamma Counter
8. Standard A as Sample Diluent (can be ordered separately)

## 10. PROCEDURE NOTES

1. Any improper handling of samples or modification of the test procedure may influence the results. The indicated pipetting volumes, incubation times, temperatures and pre-treatment steps have to be performed strictly according to the instructions. Use calibrated pipettes and devices only.
2. Once the test has been started, all steps should be completed without interruption. Make sure that required reagents, materials and devices are prepared ready at the appropriate time. Allow all reagents and specimens to reach room temperature (18 - 25°C) and gently swirl each vial of liquid reagent and sample before use. Mix reagents without foaming.
3. Avoid contamination of reagents, pipettes and wells/tubes. Use new disposable plastic pipette tips for each component and specimen. Do not interchange caps. Always cap not used vials. Do not reuse wells/tubes or reagents.
4. It is advised to perform duplicate measurements to be able to identify potential pipetting errors.
5. Labelling of all tubes is recommended.
6. The relative centrifugal force (g) is not equivalent to rounds per minute (rpm) but it has to be calculated depending on the radius of the centrifuge.

## 11. PRE-TEST SETUP INSTRUCTIONS

### 11.1. Preparation of lyophilized components

| Dilute / dissolve | with | Diluent | Remarks | Storage | Stability |
|---|---|---|---|---|---|
| **TRACER LYO** | 3.6 mL | **BUF** | **Prepare at least 30 min before use.**<br>Mix without foaming.<br>Turbid solution. | 2 - 8°C<br>Do not freeze after reconstitution. | 14 days |

### 11.2. Dilution of Samples

Positive patient sera show an individual behaviour upon dilution. With increasing concentration of autoantibodies the measured values reach a non-linear plateau. This may lead to irreconcilable results in samples that have not been diluted appropriately. Therefore it is necessary to test the linearity of dilution for each individual positive sample. It is recommended to assay all unknown samples undiluted. Samples with values above 1.5 nmol/L should be diluted with zero standard A (e.g. 1:10) and reassayed. The values of the diluted samples must be within the linear range of the assay (0.25 to 1.5 nmol/L) to give the correct test result. For patient follow up control it is appropriate to use always the same dilutions for samples from the same patient.

## 12. TEST PROCEDURE

| | |
|---|---|
| **1.** | **Pipette 20 µL** of each **Standard, Control and sample** into the respective tubes.<br>Leave two tubes empty for Total Activity (T).<br>**Note:** For a **qualitative determination**, only controls **(Standard A as Negative Control, Cut-off and Positive Control)** and **serum samples** are pipetted. |
| **2.** | **Pipette 100 µL** of **ARAb $^{125}$I-Tracer** into each tube. Mix on a vortex mixer. Set T aside. |
| **3.** | **Incubate 2 hours at room temperature (18 - 25°C).** |
| **4.** | **Pipette 200 µL** of **IgG Antiserum (Shake before use!)** into each tube (except T). Vortex |
| **5.** | **Incubate 30 minutes at room temperature (18 - 25°C).** |
| **6.** | **Pipette 1 mL** of **Wash Buffer** into each tube (except T). Vortex |
| **7.** | **Centrifuge** all tubes for **15 minutes** at 2000 - 3000 x g (refrigerated centrifuge recommended). |
| **8.** | **Decant** or aspirate the tubes carefully (except T). Do not touch the pellet. |
| **9.** | **Pipette 1 mL** of **Wash Buffer** into each tube (except T). |
| **10.** | **Resuspend** the pellets using a vortex mixer for at least **20 seconds.** |
| **11.** | **Centrifuge** the tubes again **for 15 minutes** at 2000 - 3000 x g. |
| **12.** | **Decant** or aspirate the tubes carefully (except T). |
| **13.** | **Count** the tubes in a Gamma Counter for **1 minute.** |

## 13. QUALITY CONTROL

The test results are only valid if the test has been performed following the instructions. Moreover, the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or comparable standards/laws. User and/or laboratory must have a validated system to get diagnosis according to GLP. All kit controls must be found within the acceptable ranges as stated on the labels and the QC certificate. If the criteria are not met, the run is not valid and should be repeated. Each laboratory should use known samples as further controls.

In case of any deviation the following technical issues should be proven: Expiration dates of (prepared) reagents, storage conditions, pipettes, devices, incubation conditions and washing methods.

It is recommended to participate at appropriate quality assessment trials.

## 14. CALCULATION OF RESULTS

Calculate the percent binding % B/T (% Binding/Total) for each standard as follows:

$$\% \text{ B/T} = \frac{\text{Mean cpm (Standard) x 100}}{\text{Total Activity}}$$

The obtained %B/T of the standards (y-axis, linear) are plotted against their concentration (x-axis, logarithmic) either on semi-logarithmic graph paper or using an automated method. A good fit is provided with cubic spline, 4 Parameter Logistics or Logit-Log.

For the calculation of the standard curve, apply each signal of the standards (one obvious outlier of duplicates might be omitted and the more plausible single value might be used).

The concentration of the samples can be read directly from the standard curve.

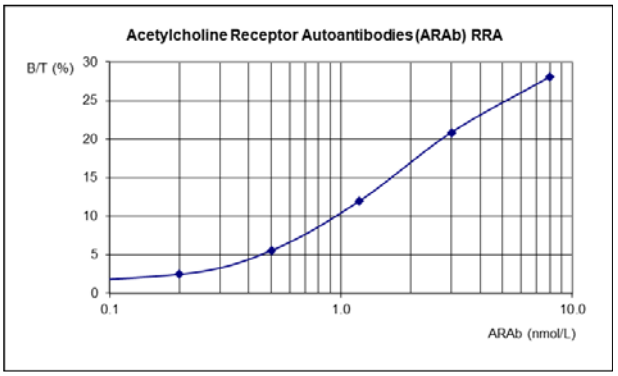
In case of diluted samples the values have to be multiplied with the corresponding dilution factor.

Samples showing concentrations above 1.5 nmol/L have to be diluted as described in chapter PRE-TEST SETUP INSTRUCTIONS and reassayed.

**Typical Calibration Curve**

(Example. Do not use for calculation!)

| Standard | ARAb | Mean | B/T |
|---|---|---|---|
| A | 0 nmol/L | 400.0 cpm | 0.48 % |
| B | 0.2 nmol/L | 2442.3 cpm | 2.44 % |
| C | 0.5 nmol/L | 5042.6 cpm | 5.54 % |
| D | 1.2 nmol/L | 10404.1 cpm | 11.94 % |
| E | 3.0 nmol/L | 17866.1 cpm | 20.85 % |
| F | 8.0 nmol/L | 23895.4 cpm | 28.05 % |




**Measuring Range:** 0.07 nmol/mL (LoQ as functional sensitivity) - 8.0 nmol/mL (Standard F).

## 15. INTERPRETATION OF RESULTS

| ARAb | Interpretation |
|---|---|
| < 0.25 nmol/L | negative |
| 0.25 - 0.4 nmol/L | equivocal |
| > 0.4 nmol/L | positive |

The results themselves should not be the only reason for any therapeutic consequences. They have to be correlated to other clinical observations and diagnostic tests.

## 16. EXPECTED VALUES

Serum and EDTA plasma samples from a total of (130/129) apparently healthy volunteers were investigated (see table below). Range of normal healthy blood donors shows values between 0.013 and 0.116 nmol/L for serum samples, for EDTA plasma samples between 0.013 and 0.086 nmol/L. The upper limit of the reference range (normal range) is at 0.110 nmol/L for serum samples and for EDTA plasma samples 0.081 nmol/L (99 % percentile). Antibody titer above 0.4 nmol/L are unambiguously indicative for myasthenia gravis.[11] Values between 0.25 and 0.4 nmol/L should be considered as equivocal. Using these values we found 93.3 % specificity and 100 % sensitivity (For more details see PERFORMANCE).

| | Mean | SD | 95 % percentile | 99 % percentile | n |
|---|---|---|---|---|---|
| Serum | 0.029 nmol/L | 0.018 nmol/L | 0.060 nmol/L | 0.110 nmol/L | 130 |
| Plasma (EDTA) | 0.023 nmol/L | 0.013 nmol/L | 0.048 nmol/L | 0.081 nmol/L | 129 |

It is recommended that each laboratory establishes its own range of normal values.

## 17. LIMITATIONS OF THE PROCEDURE

Specimen collection and storage have a significant effect on the test results. See SPECIMEN COLLECTION AND STORAGE for details.
For cross-reactivities, see PERFORMANCE.

| | | |
|---|---|---|
| The following blood components do not have a significant effect (+/- 20 % of expected value) on the test results up to the stated concentrations. | Hemoglobin | 4.00 mg/mL |
| | Bilirubin | 0.50 mg/mL |
| | Triglyceride | 30.00 mg/mL |

## 18. PERFORMANCE

**Analytical Specificity (Cross Reactivity)**
All cross reactive samples showed negative results for Myastenia gravis.
No cross-reactivity was found to:
anti-Sm-Ab, RNP-Ab, Ro (SS-A)-Ab, La (SS-B)-Ab, dsDNA-Ab, RF-Ab, ANA-Ab.

**Analytical Sensitivity (Limit of Blank - LoB)**
The LoB study was conducted in one day testing by one operator. The runs were performed with kit controls and with the zero calibrator (Standard A). The zero calibrator was tested twenty times using one reagent lot.
Limit of Blank = 0.01 nmol/L.

**Functional Sensitivity (Limit of Quantitation - LoQ)**
The LoQ study was conducted during one day testing by one operator. The runs were performed with kit controls and five different low concentrated serum samples. Each sample was tested twelve times using one reagent lots.
Limit of Quantitation = 0.07 nmol/L (with an accuracy of 20 %).

**Metrological Traceability**
The ARAb RRA is metrological traceable to SI units nmol/L by using reference data obtained from a FDA approved and accepted method comparison with the Acetylcholine Receptor Antibody (AChRAb) RIA, which is commercially available, with reference samples for ACR Antibody from EQAS according to EN ISO 17511. The uncertainty is 14.4 % according to the GUM (Guide to the expression of uncertainty in measurement) Method.
The commutability of reference material for serum samples is supported by the good, correlation between the chosen reference procedure and DiaAsource ARAb RRA (REF KIPIB21021) with r = 0.99.
Table: Passing-Bablok analysis and Pearson's coefficient

| | | |
|---|---|---|
| Y (DiaAsource ARAb RRA) = 0.8878 x Competitor Assay AChRAb RIA - 0.07394 | Y = m x X + b | r = 0.99 |

**Precision**

The intra-assay study was conducted during two days using one reagent lot. Two runs were performed with kit controls and with a panel of 7 serum samples. Each sample was tested 22 times.

| **Sample** | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| **Mean** | 0.19 nmol/L | 0.41 nmol/L | 0.75 nmol/L | 1.55 nmol/L | 2.62 nmol/L | 4.57 nmol/L | 5.72 nmol/L |
| **SD** | 0.01 nmol/L | 0.01 nmol/L | 0.02 nmol/L | 0.06 nmol/L | 0.17 nmol/L | 0.39 nmol/L | 0.65 nmol/L |
| **CV** | 5.1 % | 3.1 % | 2.8 % | 3.8 % | 6.3 % | 8.5 % | 11.3 % |

The Intra-Assay precision showed a mean CV from 5.8 % and a range of 2.8 - 11.3 % for the seven different serum samples.

The inter-assay study was conducted using one reagent lot. 15 runs were performed with kit controls and with a panel of seven serum samples. Each sample was tested in duplicate.

| **Sample** | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| **Mean** | 0.21 nmol/L | 0.40 nmol/L | 0.75 nmol/L | 1.61 nmol/L | 2.63 nmol/L | 4.72 nmol/L | 6.10 nmol/L |
| **SD** | 0.01 nmol/L | 0.01 nmol/L | 0.02 nmol/L | 0.07 nmol/L | 0.20 nmol/L | 0.59 nmol/L | 0.80 nmol/L |
| **CV** | 5.3 % | 2.8 % | 3.0 % | 4.3 % | 7.7 % | 12.4 % | 13.1 % |

The Inter-Assay precision showed a mean CV from 7.0% and a range of 2.8 – 13.1% for the seven different serum samples.

The between-lot study was conducted using 20 reagent lots. 20 runs were performed with kit controls and with a panel of five serum samples. Each sample was tested in duplicate.

| **Sample** | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| **Mean** | 0.35 nmol/L | 1.17 nmol/L | 0.69 nmol/L | 1.32 nmol/L | 12.57 nmol/L |
| **SD** | 0.02 nmol/L | 0.09 nmol/L | 0.06 nmol/L | 0.14 nmol/L | 1.45 nmol/L |
| **CV** | 5.6 % | 7.9 % | 9.3 % | 10.5 % | 11.5 % |

The between lot variation showed a mean CV from 9.0 % and a range of 5.6 - 11.5 % for the five different serum samples.

**Linearity**

The linearity study was conducted during one day of testing by one operator. The runs were performed with kit controls and with three different serum samples with different acetylcholine receptor antibody levels and diluted with zero calibrator (Standard A). Each sample was tested in duplicate using one reagent lot. The linear range as well as the plateau-range differ for each individual serum sample. Sera tested with the DiaAsource ARAb RRA were linear between 0.25 nmol/L up to 1.5 nmol/L. All sera with a concentration above 1.5 nmol/L should be diluted in order to facilitate exact quantification.
Mean Range: 101 % (81 - 126 %)
Serial dilution up to 1:64

**Recovery**

The recovery study was conducted during one day of testing by one operator. The runs were performed with kit controls and with three different serum samples which are spiked with different concentrations of acetylcholine receptor antibody. Each sample was tested in duplicate using one reagent lot.
Spiking range: 0.22 - 1.80 nmol/L
Mean recovery: 106 % (87 – 153 %)

**Clinical sensitivity / Specificity**

1. Reference intervals
The reference range (normal range) is 0.013 - 0.116 nmol/L for serum samples and for EDTA plasma samples 0.013 - 0.086 nmol/L.

2. Diagnostic Sensitivity
The diagnostic sensitivity was assessed by measuring 38 samples positive for Myasthenia gravis and calculated to be 100 %.

3. Diagnostic Specificity
The diagnostic specificity was assessed by measuring 15 samples negative for Myasthenia gravis and calculated to be 93.3 %.

4. Cut-off Index
Antibody titer above 0.4 nmol/L are unambiguously indicative for myasthenia gravis.[11] Values between 0.25 and 0.4 nmol/L should be considered as equivocal.

**Method Comparison**
The results of thirty-eight MG-positive and fifteen samples negative for MG were studied using the method of linear regression. Results from measuring the serum samples in the Predicate RIA and DiaAsource RRA yielded a correlation of $R^2 = 0.9366$ with a regression formula of: DiaAsource-Assay = 0.703 x (RIA) - 0.181; N = 53; R = 0.967

**Comparison of Serum – Plasma samples**
27 patient samples each patient serum and EDTA plasma samples was determined in DiaAsource ARAb RRA, no significant difference could be found. The correlation coefficient of the linear regression was found to be R = 0.984. The regression line was calculated to: Plasma = 0.931 x (Serum) + 0.062

## 19. PRODUCT LITERATURE REFERENCES


[1] **Gilhus, N. E., & Verschuuren, J. J. (2015).** Myasthenia gravis: subgroup classification and therapeutic strategies. The Lancet. Neurology, 14(10), 1023–1036. https://doi.org/10.1016/S1474-4422(15)00145-3

[2] **Binks, S., Vincent, A., & Palace, J. (2016).** Myasthenia gravis: a clinical-immunological update. Journal of neurology, 263(4), 826–834. https://doi.org/10.1007/s00415-015-7963-5

[3] **Farmakidis, C., Pasnoor, M., Dimachkie, M. M., & Barohn, R. J. (2018)**. Treatment of Myasthenia Gravis. Neurologic clinics, 36(2), 311–337. https://doi.org/10.1016/j.ncl.2018.01.011

[4] **Vincent, A., & Newsom-Davis, J. (1985).** Acetylcholine receptor antibody as a diagnostic test for myasthenia gravis: results in 153 validated cases and 2967 diagnostic assays. Journal of neurology, neurosurgery, and psychiatry, 48(12), 1246–1252. https://doi.org/10.1136/jnnp.48.12.1246

[5] **Nicolle M. W. (2016).** Myasthenia Gravis and Lambert-Eaton Myasthenic Syndrome. Continuum (Minneapolis, Minn.), 22(6, Muscle and Neuromuscular Junction Disorders), 1978–2005. https://doi.org/10.1212/CON.0000000000000415

[6] **Poulas, K., Koutsouraki, E., Kordas, G., Kokla, A., & Tzartos, S. J. (2012)**. Anti-MuSK-and anti-AChR-positive myasthenia gravis induced by d-penicillamine. Journal of Neuroimmunology, 250 (1-2), 94-98.

[7] **Li, Mingqiang, et al.** "Clinical characteristics of AChRAb and MuSKAb double seropositive myasthenia gravis patients." Clinical neurology and neurosurgery 172 (2018): 69-73.

[8] **Huda, S., Woodhall, M. R., Vincent, A., & Heckmann, J. M. (2016).** Characteristics Of acetylcholine-receptor-antibody–negative myasthenia gravis in a South African cohort. Muscle & nerve, 54(6), 1023-1029.

[9] **Lazaridis, K., & Tzartos, S. J. (2020).** Autoantibody specificities in myasthenia gravis; implications for improved diagnostics and therapeutics. Frontiers in Immunology, 11, 212.

[10] **Wang, S., Breskovska, I., Gandhy, S., Punga, A. R., Guptill, J. T., & Kaminski, H. J. (2018).** Advances in autoimmune myasthenia gravis management. Expert review of neurotherapeutics, 18(7), 573–588. https://doi.org/10.1080/14737175.2018.1491310

[11] **Thomas, L. (Ed.). (2020).** Labor und Diagnose: Indikation und Bewertung von Laborbefunden für die medizinische Diagnostik. TH-Books-Verlag-Ges.

# Symbols / Symbole / Symboles / Símbolos / Simboli / Símbolos / Σύμβολα

| | |
|---|---|
| REF | Cat.-No.: / Kat.-Nr.: / No.- Cat.: / Cat.-No.: / N.–Cat.: / N.º Cat.: / Αριθμός-Κατ.: |
| LOT | Lot-No.: / Chargen-Bez.: / No. Lot: / Lot-No.: / Lotto n.: / Lote N.º: / Αριθμός -Παραγωγή: |
| | Use by: / Verwendbar bis: / Utiliser à: / Usado por: / Da utilizzare entro:/ Usar até: / Χρησιμοποιείται από: |
| Σ | No. of Tests: / Kitgröße: / Nb. de Tests: / No. de Determ.: / Quantità dei tests: / N.º de Testes: / Αριθμός εξετάσεων: |
| CONC | Concentrate / Konzentrat / Concentré / Concentrar / Concentrato / Concentrado / Συμπύκνωμα |
| LYO | Lyophilized / Lyophilisat / Lyophilisé / Liofilizado / Liofilizzato / Liofilizado / Λυοφιλιασμένο |
| IVD | In Vitro Diagnostic Medical Device / In-vitro-Diagnostikum / Appareil Médical pour Diagnostics In Vitro / Dispositivo Médico para Diagnóstico In Vitro / Dispositivo Medico Diagnostico In vitro / Equipamento Médico de Diagnóstico In Vitro / Ιατρική συσκευή για In-Vitro Διάγνωση |
| BIO | Contains biological material of human origin / Enthält biologisches Material menschlichen Ursprungs / Contient une substance biologique d'origine humaine / Contiene material biológico de origen humano / Contiene materiale biologico di origine umana / Contém material biológico de origem humana / Περιέχει βιολογικό υλικό ανθρώπινης προέλευσης |
| BIO | Contains biological material of animal origin / Enthält biologisches Material tierischen Ursprungs / Contient une substance biologique d'origine animale / Contiene material biológico de origen animal / Contiene materiale biologico di origine animale / Contém material biológico de origem animal / Περιέχει βιολογικό υλικό ζωικής προέλευσης |
| UDI | Unique Device Identification / Eindeutige Gerätekennung / Identifiant de dispositif unique / Identificación única de producto / Identificatore univoco del dispositivo / Identificador de dispositivo único / Μοναδικός αναγνωριστικός κωδικός προϊόντος |
| Radioaktiv | Radioactive / Radioaktiv / Radioactif / Radiactivo / Radioattivo / Radioactivo / Ραδιενεργό. |
| | Read instructions before use / Arbeitsanleitung lesen / Lire la fiche technique avant emploi / Lea las instrucciones antes de usar / Leggere le istruzioni prima dell'uso / Ler as instruções antes de usar / Διαβάστε τις οδηγίες πριν την χρήση |
| | Keep away from heat or direct sun light / Vor Hitze und direkter Sonneneinstrahlung schützen / Garder à l'abri de la chaleur et de toute exposition lumineuse / Manténgase alejado del calor o la luz solar directa / Non esporre ai raggi solari / Manter longe do calor ou luz solar directa / Να φυλάσσεται μακριά από θερμότητα και άμεση επαφή με το φως του ηλίου |
| | Store at: / Lagern bei: / Stocker à: / Almacene a: / Armazenar a: / Conservare a: / Armazenar em: / Αποθήκευση στους: |
| 2-8°C | Store at: 2 - 8°C / Lagern bei: 2 - 8°C / Stocker à: 2 - 8°C / Almacene a: 2 - 8°C / Armazenar a: 2 - 8°C / Conservare a: 2-8°C / Armazenar em: 2-8°C / Αποθήκευση στους: 2-8°C |
| | Manufacturer: / Hersteller: / Fabricant: / Productor: / Fabricante: / Fabbricante: / Παραγωγός: |
| | Distributor: / Distributor: / Distributeur: / Distributor: / Distributore: / Distribuidor: / Διανομέας: |
| | Caution! / Vorsicht! / Attention! / ¡Precaución! / Attenzione! / Cuidado! / Προσοχή! |
| | Symbols of the kit components see MATERIALS SUPPLIED.<br>Die Symbole der Komponenten sind im Kapitel KOMPONENTEN DES KITS beschrieben.<br>Voir MATERIEL FOURNI pour les symbôles des composants du kit.<br>Símbolos de los componentes del juego de reactivos, vea MATERIALES SUMINISTRADOS.<br>Per i simboli dei componenti del kit si veda COMPONENTI DEL KIT.<br>Para símbolos dos componentes do kit ver MATERIAIS FORNECIDOS.<br>Για τα σύμβολα των συστατικών του κιτ συμβουλευτείτε το ΠΑΡΕΧΟΜΕΝΑ ΥΛΙΚΑ. |

Generic table, not all symbols are present in the product

COMPLAINTS: Complaints may be submitted initially written or vocal. Subsequently they need to be filed including the test performance and results in writing in case of analytical reasons.

WARRANTY: The product is warranted to be free from material defects within the specific shelf life and to comply with product specifications delivered with the product. The product must be used according to the Intended use, all instructions given in the instructions for use and within the product specific shelf life. Any modification of the test procedure or exchange or mixing of components of different lots could negatively affect the results. These cases invalidate any claim for replacement.

LIMITATION OF LIABILITY: IN ALL CIRCUMSTANCES THE EXTENT OF MANUFACTURER'S LIABILITY IS LIMITED TO THE PURCHASE PRICE OF THE KIT(S) IN QUESTION. IN NO EVENT SHALL MANUFACTURER BE LIABLE FOR ANY INCIDENTAL OR CONSEQUENTIAL DAMAGES, INCLUDING DAMAGES FOR LOST PROFITS, LOST SALES, INJURY TO PERSON OR PROPERTY OR ANY OTHER INCIDENTAL OR CONSEQUENTIAL LOSS.

The labelling of hazardous substances is according to European directive.
For further country-specific classifications, please refer to the corresponding safety data sheet.

**IBL International GmbH**
Flughafenstrasse 52a
22335 Hamburg, Germany
Phone: +49 (0)40-53 28 91-0
Fax: +49 (0)40-53 28 91-11
IBL@tecan.com
www.tecan.com/ibl

Always there for you